Enhancement of Platelet Deposition by Cross-Linked Hemoglobin in a Rat Carotid Endarterectomy Model

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Background Purified human cross-linked hemoglobin, which is now being used in clinical trials, increases mean arterial pressure through binding of nitric oxide (NO). We postulated that binding of NO by cross-linked hemoglobin ($\alpha\alpha$Hb) could also increase platelet deposition at sites of subintimal injury.

Methods and Results Male Sprague-Dawley rats were infused with $\alpha\alpha$Hb (0.88 g/kg, n=8) or with the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA, 30 mg/kg, n=7) before undergoing microsurgical carotid endarterectomy. ^{111}In-labeled platelets were infused after endarterectomy, and platelet deposition was measured 20 minutes later. In control endarterectomized rats (n=8), mean platelet deposition was $7.7\pm0.7\times10^6$/mm^2. Platelet deposition was significantly increased above controls in rats that received $\alpha\alpha$Hb ($13.2\pm0.9\times10^6$/mm^2, P=.0004) and in rats infused with L-NMMA ($13.9\pm1.0\times10^6$/mm^2, P=.0002). The increase was prevented by infusion of L-arginine (150 mg/kg) immediately after $\alpha\alpha$Hb or L-NMMA. To determine whether aspirin (ASA) blocked the increased deposition induced by $\alpha\alpha$Hb, rats received oral ASA (10 mg/kg) 18 hours before endarterectomy. Platelet deposition in animals receiving ASA alone was $6.4\pm0.9\times10^6$/mm^2 (n=8). This was significantly increased to $10.8\pm0.8\times10^6$/mm^2 (P=.002) for the ASA-treated group that received $\alpha\alpha$Hb at the time of endarterectomy (n=8). The prolonged bleeding times induced by ASA were unaffected by the infusion of $\alpha\alpha$Hb.

Conclusions These data suggest that in a rat endarterectomy model, $\alpha\alpha$Hb increases platelet deposition at sites of subintimal injury by binding NO. Increased deposition induced by $\alpha\alpha$Hb can be prevented by administration of L-arginine but not by pretreatment with aspirin. (***Circulation.*** 1996;93: 327-332.)

Key Words • hemoglobin • nitric oxide • platelets • surgery

Several different preparations of purified human hemoglobin, cross-linked to prevent renal damage, are currently undergoing clinical trials as an oxygen-carrying agent.[1] In addition to binding oxygen, hemoglobin is known to have a very high affinity for NO.[2,3] Infusion of hemoglobin into animals increases blood pressure,[4-8] at least in part because of binding of NO to hemoglobin, which also results in inhibition of NO-mediated vasodilation.

In addition to moderating blood pressure, NO inhibits platelet function through increasing levels of platelet cGMP.[9-13] Several studies suggest that this interaction can be modulated by hemoglobin. Incubation of hemoglobin with monolayers of bovine endothelial cells promotes platelet adhesion,[9] and platelets obtained from rabbits that have been infused with hemoglobin demonstrate an increased response to agonists ex vivo, as measured by aggregometry.[13] In vivo studies have not been done to determine whether hemoglobin, by binding to NO, can promote platelet deposition at the site of endothelial injury. We therefore investigated the effects of $\alpha\alpha$Hb on platelet deposition in rats undergoing microsurgical carotid endarterectomy.

See p 208

Methods

Reagents

The NO synthase inhibitor L-NMMA was obtained from Calbiochem-Novabiochem Corp and was administered intravenously in 0.5 mL normal saline. L-Arg (Sigma Chemical Co) was administered intravenously in 1.5 mL normal saline. Generic ASA powder (Mallinckrodt Chemical Works) was dissolved at a concentration of 10 mg/mL in polyethylene glycol (average molecular mass, 4 kD; Sigma Chemical Co).

Cell-free human hemoglobin, lot 91196, was produced at Letterman Army Institute of Research from outdated red blood cells by methods previously described.[14] Final concentration of the hemoglobin solution was 7.0 g/dL. The hemoglobin was cross-linked between α-chains with bis(3,5-dibromosalicyl)-fumarate, preserved in Ringer's acetate, divided into aliquots, and frozen at −80°C until the day of use.

The endotoxin concentration, as determined by a kinetic turbidometric assay using limulus amebocyte lysate,[14] was 0.25 endotoxin units/mL. Two samples of this preparation of hemoglobin ($\alpha\alpha$Hb; molecular mass, 64 kD) passed the rabbit pyrogenicity test as administered at the Center for Biologics Evaluation and Research (Food and Drug Administration). The solution had no bacterial contamination, as shown by a sterility test conducted over a period of 1 week. The concen-

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Selected Abbreviations and Acronyms

αα Hb = purified human cross-linked hemoglobin
ASA = acetylsalicylic acid (aspirin)
L-Arg = L-arginine
L-NMMA = N^G-monomethyl-L-arginine
MAP = mean arterial pressure
NO = nitric oxide
SIN-1 = 3-morpholinosydnonimine

tration of methemoglobin was 2.6%, and the pH was 7.35. The P_{50} was 25 mm Hg.

Assay Procedures

Plasma hemoglobin was measured with the Radiometer OSM 3 Hemoximeter. Platelets were enumerated in the Baker Hematology Series Cell Counter System 9000.

Animals

Male Sprague-Dawley rats (mean weight, 434 g) were allowed ad libitum access to water and standard rat chow until the time of anesthesia. Animal procedures were approved by the Walter Reed Army Institute of Research Laboratory Animal Care and Use Committee and were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*, NIH publication 86-23, revised 1985.

Preparation of Donor Platelets

Homologous platelets for each experiment were obtained from a separate donor rat, which was first anesthetized with pentobarbital (50 mg/kg IP). A femoral artery catheter was then inserted, and the donor rat was exsanguinated. Platelets from 12 mL whole blood (anticoagulated with 2 mL acid citrate dextrose) were labeled with 100 μCi ^{111}In oxine (^{111}In, MPI Pharmacy Inc) by a modification of the labeling technique described by Bauman and Landry.[15] Briefly, platelet-rich plasma was centrifuged at 300*g* for 10 minutes, the supernatant was decanted, and the platelets were resuspended in acid citrate dextrose anticoagulant. ^{111}In was then incubated with the platelet suspension for 30 minutes at 22°C. The platelets were then washed and resuspended in 2 mL native platelet-poor plasma. Mean labeling efficiency was 84±7% (mean±SD).

Experimental Protocol

The animal model, which has been described previously,[16] is a modification of the microsurgical carotid endarterectomy model developed by Sasaki et al.[17] The experimental rats were initially anesthetized with pentobarbital at a dose of 50 mg/kg IP, with subsequent doses (20 mg/kg IP) used as necessary to maintain adequate anesthesia. Surgical procedures were performed under ×5 to ×20 magnification with a Zeiss OPMI 6 series operating microscope (Carl Zeiss, Inc, Medical Products Division).

Fig 1 outlines the experimental protocol. The experimental rat was secured, and a catheter was placed in the femoral vein. Normal saline was infused through this line at a maintenance rate of 2.75 $mL \cdot kg^{-1} \cdot h^{-1}$ with a model 355 Orion syringe pump (Orion Research Inc). A femoral artery catheter was then placed, and arterial pressure was monitored through a transducer with a Datascope 2002A hemodynamic monitor. The initial MAP was recorded, and then blood (2 mL) was withdrawn from the arterial line for measurement of the platelet count and plasma hemoglobin level. The blood was replaced by an equal volume of normal saline. An initial bleeding time was then determined by methods described below. A preinfusion MAP was recorded, and group-specific reagents (L-NMMA or αα Hb) were then administered intravenously. The dose of αα Hb administered (1.25 mL/100 g body wt, or 0.88 g αα Hb/kg) was equivalent to 10% of the total

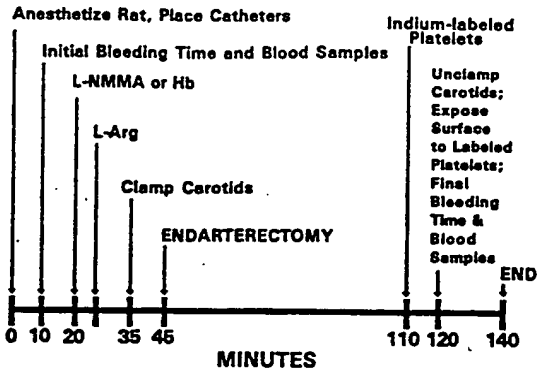

FIG 1. Time line for rat carotid endarterectomy model. The initial bleeding time was measured in the anesthetized rat before the infusion of any study agents and then repeated 20 minutes before the termination of the experiment. The study agents were infused more than 1 hour before the endarterectomy procedure.

hemoglobin in the rat. The αα Hb was infused through the venous catheter during 5 minutes, with simultaneous withdrawal of an equal amount of blood from the arterial catheter to avoid volume changes. Postinfusion MAPs were recorded 2 minutes after the completion of each infusion.

The trachea and both common carotid arteries were exposed through a midline cervical incision, and a tracheostomy tube was placed. The right (experimental side) and left (sham-operated side) common carotid arteries were isolated and clamped with low-pressure Kleinert microvascular clamps. Two-millimeter longitudinal arteriotomies were made in both the experimental and sham-operated arteries. Stay sutures of 10-0 nylon were used to expose the lumen of the experimental carotid artery, and an endarterectomy of the back wall was performed by placement of two transverse and parallel score marks in the intima 0.5 mm apart with the point of a 27-gauge needle and removal of the intervening intima with microforceps, exposing the vessel media. Both arteriotomies were then closed with running 10-0 nylon sutures.

^{111}In-labeled platelets prepared from the donor rat were given intravenously (1.5 mL volume) after arteriotomy closures. MAP was recorded 2 minutes after the platelet infusion. Platelets were allowed to circulate for 10 minutes before removal of the arterial clamps and exposure to the carotid endarterectomy site for 20 minutes until termination of the experiment. A bleeding time was obtained at the beginning of platelet circulation time, and 2 mL of blood was then withdrawn from the arterial catheter for repeat determination of the platelet count and plasma hemoglobin level.

After the 20-minute circulation time, a final MAP was recorded. The animal was hemodiluted by withdrawal of blood from the arterial catheter and injection of saline into the venous catheter and was perfusion-fixed with the injection into the arterial line of 20 mL 4% formaldehyde/1% glutaraldehyde. Three-millimeter sections of both carotid arteries that contained the suture lines were excised and placed in fixative. Occlusive thrombi were not present in the carotid arteries of any of the experimental animals.

Bleeding Time

After excision of the distal 1 mm of the tail, a cotton-tipped applicator was gently applied to the wound every 30 seconds for the first 5 minutes and every 10 seconds thereafter until cessation of oozing. The time at which blood was no longer evident on the swab was taken as the bleeding time.

^{111}In-Labeled Platelet Counting

The radioactivity of the experimental and sham carotid specimens and of blood samples was determined in a T

Analytic Gamma Trac 1193 gamma well-counter programmed for ^{111}In photon peak.

Endarterect my Area Measurement

After measurement f radioactivity, the experimental carotid artery was opened longitudinally through the arteriotomy suture line and mounted on a silicon rubber block with minutien pins. The specimen was postfixed with osmium tetroxide, critical point–dried with carbon dioxide, and coated with gold palladium. The artery was then examined with scanning electron microscopy at ×25 to ×50 magnification, and an electron photomicrograph that contained the entire endarterectomized segment was generated. This photomicrograph was analyzed with computer image analysis software (Optimus Bioscan Inc), and the surface area of the endarterectomy was determined.

Primary Outcome Measure

The effect of the experimental treatments on platelet deposition was measured as the number of adherent ^{111}In-labeled platelets per square millimeter of the carotid endarterectomy. The radioactivity of whole blood (cpm/μL) and platelet count were measured just before euthanasia, and the specific activity of circulating platelets (cpm/platelet) was calculated. The ^{111}In-labeled platelet activity per square millimeter of the endarterectomy site was calculated by subtracting the activity of the sham specimen from the activity of the experimental specimen and dividing the net radioactivity by the area (in square millimeters) of the endarterectomy sites. Platelet deposition, defined as total platelets per square millimeter on the endarterectomy site was computed as platelet deposition (platelets/mm^2)=cpm/mm^2×platelets/cpm.

Experimental Groups

The experimental protocol was performed in two series. Series 1 (n=40) consisted of six experimental groups: (1) control (n=8); (2) infusion of L-NMMA alone (30 mg/kg, n=7); (3) infusion of L-NMMA followed by L-Arg (150 mg/kg, n=7); (4) infusion of $\alpha\alpha$Hb alone (0.88 g/kg, n=7); (5) infusion of $\alpha\alpha$Hb followed by L-Arg (n=7); and (6) infusion of L-Arg alone (n=4).

Series 2 (n=16) included two additional experimental groups: (1) oral administration of ASA alone (10 mg/kg, n=8) and (2) oral administration of ASA followed by intravenous $\alpha\alpha$Hb (0.88 g/kg). ASA was administered orally through a gastric tube the evening before the experiment to both donor and experimental animals, and $\alpha\alpha$Hb was administered before the endarterectomy as in series 1. In each series, to control for possible time effects, block randomization was used to assign animals to experimental groups. For series 1, each block contained 6 rats (1 from each experimental group), and for series 2, each block contained 2 rats (1 from each of the two experimental groups).

Exclusions

A total of 59 rats underwent carotid endarterectomy. Three rats died during the surgical procedure and were excluded from the analysis. One rat developed copious pulmonary secretions and respiratory distress after platelet administration, and 1 rat became hypotensive after $\alpha\alpha$Hb and L-Arg infusion. The third rat, a recipient of L-NMMA and L-Arg, developed hypotension and died after the carotid arteries were unclamped.

Statistical Analysis

Summary results for continuous outcomes are reported as mean±SEM. ANOVA for independent groups was used to determine the overall statistical significance of differences in mean platelet deposition.[18,19] Analysis was done on transformed (square root and rank) and untransformed data with similar results. The unpaired *t* test was used for individual comparisons in mean platelet deposition between two groups

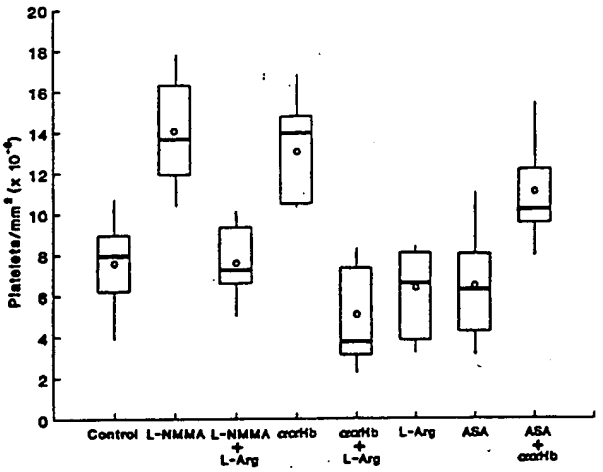

FIG 2. Platelet deposition at the site of the carotid endarterectomy, as assessed with ^{111}In-labeled platelets, is shown as a box plot for the controls and each of the experimental groups. For each group, the mean platelet deposition is shown by the circle and the median by the horizontal line within the box. The horizontal lines at the top and bottom of each box represent the upper and lower quartiles. The vertical line at the top extends to the highest data point; the vertical line at the bottom extends to the lowest data point. Compared with the control group, platelet deposition was significantly increased in the groups receiving L-NMMA (P=.0002), $\alpha\alpha$Hb (P=.0004), and ASA followed by $\alpha\alpha$Hb (P=.016). Deposition was significantly decreased in the group receiving $\alpha\alpha$Hb and L-Arg (P=.028).

by use of the ANOVA mean square error for untransformed data. Overall differences between the initial and final bleeding times (logarithmically transformed) among groups were assessed by repeated measures ANOVA. Paired *t* tests were used for mean changes in bleeding time for a single experimental group (by repeated measures mean square error). All reported probability values are two-sided; no adjustment was made for multiple testing. A value of $P<.05$ was considered to be significant. Statistical calculations were carried out with Minitab statistical software.[20]

Results

Effect of L-NMMA and $\alpha\alpha$Hb on Subintimal Platelet Deposition

We hypothesized that in animals infused with $\alpha\alpha$Hb, platelet deposition could be increased because of binding of NO to circulating free hemoglobin. To determine whether increased platelet deposition could be detected in this animal model if NO were inhibited, one group was infused with L-NMMA, a known inhibitor of NO synthase. In control rats, mean platelet deposition at the endarterectomy site was $7.7\pm0.7\times10^6$/mm^2 (Fig 2), and in the group receiving L-NMMA, platelet deposition was significantly increased by 81% to $13.9\pm1.0\times10^6$/mm^2 (P=.0002). In the group infused with $\alpha\alpha$Hb, platelet deposition was $13.2\pm0.9\times10^6$/mm^2, an increase of 71% above controls (P=.0004).

L-Arg, a substrate for NO synthase, was infused immediately after L-NMMA or $\alpha\alpha$Hb to determine whether sufficient NO could be generated to prevent increased platelet deposition. In one group that received L-Arg alone, platelet deposition was less than that of the control group, although the reduction was not significant ($6.1\pm1.1\times10^6$/mm^2 versus $7.7\pm0.7\times10^6$/mm^2, P=.42). In the group receiving $\alpha\alpha$Hb followed by L-Arg, platelet deposition was $4.8\pm0.9\times10^6$/mm^2, a value less than that

TABLE 1. Bleeding Times and Plat let Counts in Control and Experimental Groups

Group (n)	Initial Bleeding Time, s	Final Bleeding Time, s	Initial Platelet Counts, $\times10^9$/L	Final Platelet Counts, $\times10^9$/L
Control (8)	557±48	559±39	779±67	790±82
L-NMMA (7)	673±19	604±26	784±60	849±47
L-NMMA/L-Arg (7)	573±36	618±31	778±61	816±82
αα-Hb (7)	556±32	519±31	793±90	751±51
αα-Hb/L-Arg (7)	587±31	588±36	724±60	658±57
L-Arg (4)	632±24	628±35	752±62	892±55
ASA (8)	730±33*	705±35*	746±54	776±64
ASA/αα-Hb (8)	734±39*	721±31*	837±46	769±60

Values are mean±SEM.
*Value is significantly increased above that of the control group ($P<.01$).

of the control group ($P=.028$). Platelet deposition was $7.5\pm0.6\times10^6$/mm² in the group infused with L-NMMA and L-Arg and was not different from the control group ($P=.88$). Thus, infusion of L-Arg into groups that had received αα-Hb or L-NMMA successfully blocked the increase in deposition, resulting in platelet deposition that was either the same as or less than that of the control group.

Additional studies were performed to determine whether ASA blocked the increased deposition noted with αα-Hb. Two groups received ASA 18 hours before carotid endarterectomy; one group also received αα-Hb just before the surgery. In the ASA-treated group that also received αα-Hb, platelet deposition was $10.8\pm0.8\times10^6$/mm². This was significantly higher than the value of $6.4\pm0.9\times10^6$/mm² for the group receiving ASA alone ($P=.002$) and for the control group ($7.7\pm0.7\times10^6$/mm², $P=.016$). There was no difference in platelet deposition between the control group and the rats receiving ASA alone ($7.7\pm0.7\times10^6$/mm² versus $6.4\pm0.9\times10^6$/mm², $P=.28$).

Bleeding Times and Platelet Counts in Experimental Groups

Comparison of initial and final bleeding times within groups showed that for the group receiving L-NMMA, the final bleeding time was decreased by 10% from that of the initial bleeding time (673±19 versus 604±26 seconds, $P=.012$). In all other groups, including those receiving ASA alone and ASA and αα-Hb, the final bleeding time did not differ significantly from the initial bleeding time (Table 1).

A comparison of bleeding times among the groups showed that the initial bleeding time in the two groups receiving ASA was significantly higher than the initial bleeding time for the control group ($P<.01$). For the group receiving ASA followed by αα-Hb at the time of endarterectomy, the initial and final bleeding times were not different from the group receiving ASA alone ($P>.5$).

Although no significant differences in platelet counts were obtained between the beginning and end of the experimental protocol in any of the groups, platelet counts tended to be lower at the end of the experiment in those groups that received αα-Hb (Table 1).

Hemodynamic Effects of L-NMMA and of αα-Hb

In control rats, the MAP ranged from 88 to 102 mm Hg throughout the experiment (Table 2). In the group receiving αα-Hb and L-Arg, the MAP increased from 94±5 to 118±6 mm Hg after infusion of αα-Hb ($P=.0042$); after the infusion of L-Arg, the MAP was not significantly different from the baseline value ($P=.079$). Similarly, for rats that received both L-NMMA and L-Arg, MAP was significantly increased after infusion of L-NMMA ($P=.0012$), but shortly after the infusion of L-Arg, it was reduced to values that were not different from baseline ($P=.206$). Pretreatment of rats with ASA before the infusion of αα-Hb did not affect the hemodynamic response.

In the αα-Hb-treated group, the MAP measured after the infusion of ^{111}In-labeled platelets was not different from that measured at the termination of the experiment. In all other groups, the MAP decreased during this time. However, the MAP did not appear to influence platelet deposition. There was no correlation between platelet deposition and the MAP measured immediately after the infusion of ^{111}In-labeled platelets ($r=-.172$, $P=.22$) or between platelet deposition and the absolute decrease in MAP that occurred during the time between the infusion of the ^{111}In platelets and the termination of the experiment ($r=.096$, $P=.48$).

Plasma Hemoglobin Levels

In the three groups of rats receiving αα-Hb (n=22), the mean preinfusion plasma level of hemoglobin was 86±18 mg/dL. When measured at the end of the experiment (ie, 100 minutes after infusion of αα-Hb), the mean plasma hemoglobin level was 618±49 mg/dL ($P<.0001$). The mean plasma hemoglobin level for rats (n=14) not receiving αα-Hb was 71±16 mg/dL at the beginning of the

TABLE 2. MAP in Experimental Groups

Group (n)	Preinfusion L-NMMA or αα-Hb	Postinfusion L-NMMA or αα-Hb, 2 min	Postinfusion L-Arg, 2 min	Postinfusion ^{111}In-Labeled Platelets, 2 min	End Study
Control (8)	88±5	...	...	102±10	98±6
L-NMMA (7)	96±4	125±5*	...	113±7	96±6
L-NMMA/L-Arg (7)	92±5	116±6*	98±6	111±6	93±5
αα-Hb (7)	85±5	109±6*	...	96±4	96±5
αα-Hb/L-Arg (7)	94±5	118±6*	103±5	120±8	103±6
L-Arg (4)	99±3	...	99±2	118±5	93±6
ASA (8)	99±4	...	...	99±5	95±4
ASA/αα-Hb (8)	98±4	119±5*	...	112±6	107±5

Values are mm Hg (mean±SEM).
*Value is significantly increased above the corresponding preinfusion MAP ($P<.01$).

experiment and 157±89 mg/dL at the end; the increase was not statistically significant (P=.143).

Discussion

The present study demonstrates that in the rat microsurgical model, the infusion of the NO synthase inhibitor L-NMMA or αα-Hb before endarterectomy significantly increases platelet deposition. Administration of L-Arg after L-NMMA or αα-Hb abrogated this response, strengthening the association of platelet deposition with NO availability and indicating a possible role for L-Arg in modifying the αα-Hb-associated increase in platelet deposition.

Platelet deposition in ASA-treated rats infused with αα-Hb was increased above that of control rats or rats receiving ASA alone, indicating that ASA does not block the proaggregatory effects of αα-Hb. These findings are consistent with recent studies by Broekman et al,[21] who demonstrated that NO-mediated inhibition of platelet reactivity was ASA independent.

ASA caused a significant increase in bleeding time, which was sustained even with the infusion of αα-Hb. Thus, the bleeding time did not accurately reflect the increased platelet deposition at the site of the endarterectomy. These data are consistent with other reports demonstrating that the bleeding time does not predict the hemostatic function of platelets in animal models[22] or in patients subjected to surgery.[23-25]

MAPs paralleled those expected with changes in NO availability, with significant increases occurring shortly after infusion of L-NMMA or αα-Hb, followed by restoration to preinfusion values after the administration of L-Arg. Infusion of L-Arg alone caused no significant change in MAP. The effect of αα-Hb on MAP in this model was similar to that found in conscious rats.[4]

Rats received αα-Hb at a dose that was ≈10% of the whole blood hemoglobin; by the end of the experiment, the plasma values were equivalent to 5% of the total hemoglobin concentration. The relatively short half-life is consistent with that found in other studies.[5] In control rats, plasma hemoglobin levels remained at ≤1% of the total hemoglobin throughout the experiment. Similar plasma hemoglobin values have been reported in control rats in other studies; this may be due to an increased fragility of rat erythrocytes, which results in lysis either in vivo or ex vivo.[26]

The role of NO in regulating platelet function has been suggested in several in vivo and in vitro studies. In vitro studies have confirmed that NO can partially inhibit platelet aggregation[27] or platelet adhesion to endothelial cells.[9] These effects can be reversed by the addition of hemoglobin.[2,9] Hemoglobin does not appear to have a direct effect on platelet function in vitro.[27,28]

In one study, platelet deposition, as assessed by electron microscopy, was increased on injured endothelium of rabbits infused with L-NMMA.[29] Yao et al[30] found that infusion of L-NMMA into dogs with a mechanical injury and stenosis of either a coronary artery or femoral artery resulted in recurrent platelet aggregation as assessed by cyclic flow variation; the aggregation was prevented by infusion of L-Arg. Groves et al[31] performed bilateral carotid angioplasty on anesthetized pigs and compared aggregation of ^{111}In-labeled platelets between controls and animals receiving the NO donor SIN-1. Compared with controls, SIN-1 significantly reduced platelet aggregation at sites of both superficial and deep arterial injury. The use of SIN-1 was further accompanied by a significant increase in platelet cGMP.

It is unknown whether the increased platelet deposition associated with the infusion of αα-Hb is a dose-dependent phenomenon, since only one concentration of αα-Hb, which was calculated to be equivalent to a one-unit transfusion in a human recipient, was used in this study. The lowest doses at which NO will be completely inhibited have not been established. It is also unknown whether the effect of hemoglobin on platelet deposition is a property of the hemoglobin preparations that are now in clinical trials. The significance of these findings therefore remains to be investigated in human recipients of commercial products.

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